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Chapter

Target Therapy in Acute Myeloid Leukemia

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Abstract

Acute myeloid leukemia (AML) is the most common form of acute leukemia in elderly patients. Over the past four decades the basic therapeutic armamentarium was the standard cytotoxic treatment. The new insights in understanding the pathogenesis of AML was the momentum that revolutionized the treatment landscape in AML. The last five years unprecedented growth has been seen in the number of target therapy drugs for the treatment of AML. These new drugs did not just have a clinical benefit as single agents but also have improved AML patient outcomes if combined with conventional cytotoxic therapy. Here, we review recent advances in target-based therapy for patients with AML focusing on their mechanism of action and the results from already published clinical trials.

Keywords: acute myeloid leukemia, target therapy, FLT3 inhibitors, IDH inhibitors, pro-apoptotic agents, smoothened inhibitors, checkpoint inhibitors, CD33-targeted therapy, E-selectin inhibitors, Polo-like kinase inhibitors

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignant disease, characterized by uncontrolled proliferation with impaired differentiation of myeloid progenitor cells and aggressive clinical course. In the past two decades the treatment landscape of AML underwent significant changes due to explosive growth in knowledge of the molecular pathways involved in the AML origin and course evolution. This increased new data and understanding of the pathogenesis of AML, facilitated the development of new drugs in the treatment of AML, particularly the creation of drugs that target the disease on a molecular level. Encouraging efficacy of targeted therapy when combined with the traditional chemotherapy have resulted in big improvement to AML treatment and survival. In this chapter, we will discuss the drugs used in treatment of AML, including targeted treatment strategies that have recently entered the clinical practice.

1.1 Signaling and kinase pathway mutations inhibitors

1.1.1 FLT3 tyrosine kinase inhibitors

Full-length human FLT3 was cloned from a pre-B cell library in 1993 [1], from a CD34+ hematopoietic stem cell-enriched library [2], and is located on chromosome 13q12 [3]. FLT3 is a member of class III receptor tyrosine kinases (RTK) [4] and its activation leads to promotion of cell survival, proliferation, and differentiation

through various signaling pathways, including PI3K, RAS, and STAT5 [5]. It is present in approximately 20–30% of adult AML patients and 5–15% of pediatric AML patients [6, 7]. FLT3-ITD mutations are associated with higher relapse rate and poorer overall survival, particularly with a high ratio of mutant allelic burden [8, 9]. Several first- and next generation FLT3 inhibitors have been investigated in patients with FLT3-ITD-mutated AML.

1.1.1.1 First-generation FLT3 inhibitors

1.1.1.1.1 Midostaurin

Midostaurin (Rydapt®, Novartis Pharmaceuticals, Inc. (PKC412)) is a multikinase inhibitor, targets wild type FLT3 and mutated FLT3 (ITD and tyrosine kinase domain (TKD)) [10]. Midostaurin also inhibits c-kit, platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and protein kinase C [11]. A phase III international prospective, multinational, randomized, placebo-controlled, double-blind RATIFY study confirmed that, addition of midostaurin to standard induction chemotherapy could significantly increase OS vs. placebo among AML adults with FLT3 mutation (median OS of 74.7 m vs. 25.6 m, HR = 0.78, n = 717). RATIFY enrolled adults aged 18–59 years with newly diagnosed FLT3-mutated (ITD or TKD) AML. Patients were stratified according to FLT3 mutation type (TKD, ITD with a high AR [>0.7], and ITD with a low AR [0.05–0.7]) The addition of midostaurin demonstrated a significant survival benefit in patients with FLT3-mutated AML compared with placebo, with a 22% reduction in the rate of death compared with placebo (hazard ratio [HR], 0.78 [95% CI, 0.63–0.96]; P = 0.009). Although not specifically mandated, allogeneic stem cell transplantation (allo-SCT) was performed in 25% of patients in first complete remission (CR) and 57% of patients overall. Furthermore, patients receiving an allo-SCT in first CR had better outcome if they were treated with midostaurin during induction therapy (P = 0.08), suggesting that the optimal treatment strategy in FLT3-mutated AML would be to move on to allo-SCT early in first CR [12]. Adverse events (AEs) occurring during treatment with midostaurin were common in patients receiving intensive chemotherapy for AML. The most common nonhematologic grade ≥ 3 AEs were febrile neutropenia, infection, lymphopenia, diarrhea, and rash/desquamation. The rates of grade ≥ 3 AEs were largely similar between the midostaurin and placebo groups, with the exceptions of rash/desquamation and anemia (higher in the midostaurin group) and nausea (nearly twice as common in the placebo group) [12, 13]. Other, less common AEs reported with midostaurin included pulmonary AEs (i.e., pneumonitis or pulmonary infiltrate), cardiac AEs (e.g., prolonged corrected QT interval) and hepatic or renal dysfunction [12–14].

1.1.1.1.2 Sorafenib

Sorafenib is a potent first-generation multikinase inhibitor with activity against FLT3/ITD receptor but resistance emerges as FLT3-TKD point mutations [15]. It has been evaluated as either single agent or in combination with chemotherapies in numerous phase I and phase II clinical trials [16–20]. In an early phase clinical trial, sorafenib combined with idarubicin and high dose cytarabine in younger de novo AML patients provided a CR rate of 93% and a 1-year survival rate of 74% in FLT3-ITD positive AML patients [18]. In SORAML study, a placebo-controlled randomized study from Germany in 267 newly diagnosed patients aged 18–60 years. Sorafenib was added to daunorubicin and cytarabine (7 + 3) which resulted in a significantly prolonged 3-year EFS (40 vs. 22%, P = 0.013) and RFS

(56 vs. 38%, P = 0.017) without improvement in OS and CR [21]. Another randomized placebo-controlled trial in 201 older patients aged 61–80 years did not demonstrated improvement in EFS, CR, and OS. The results showed higher early mortality (17 versus 7%, P = 0.052) compared with placebo [22]. In multivariate analysis of retrospective study analysing the effect of sorafenib as post-transplant maintenance in adult patients with FLT3-internal tandem duplication (ITD) acute myeloid leukaemia (AML) in 26 sorafenib patients and 55 controls, sorafenib significantly improved OS [Hazard ratio (HR) 0·26, P = 0·021] and PFS (HR 0·25, P = 0·016). Also there was no difference in 2-year non-relapse mortality (9·8% vs. 9·3%, P = 0·82) or 1-year chronic graft-versus-host disease (55·5% vs. 37·2%, P = 0·28) [23]. The most common adverse events occurring during treatment with sorafenib in SORAML study were fever, infections, pneumonia and pain. Grade 3 or worse adverse events that were significantly more common in the sorafenib group than the placebo group were fever, diarrhea, bleeding, cardiac events, hand-foot-skin reaction and rash [21].

1.1.1.1.3 Sunitinib

Sunitinib is an oral multitargeted kinase inhibitor with selectivity for FLT3, PDGFa/b, VEGF receptor, and Kit receptor tyrosine kinases [24]. Sunitinib induces G1 phase arrest, increases pro-apoptotic molecule expression, and decreases anti-apoptotic molecule expression in AML cells [25]. In a study by O'Farrell and colleagues in 29 AML patients each received a single dose of sunitinib, inhibition of FLT3 phosphorylation was observed in 50% of FLT3 wild-type patients and 100% of FLT3 mutated patients [26]. In another phase I study of sunitinib in 15 patients with refractory AML partial responses were achieved in all 4 patients with FLT3 mutations compared with 2 of 10 in patients with wild-type FLT3. All responses were of short duration and the most frequent grade 2 toxicities were edema, fatigue, and oral ulcerations occurring with a regimen of 50 mg/week [27]. In another phase I/II clinical trial, sunitinib combined with intensive chemotherapy included 22 patients older than 60 years with FLT3/ITD-mutated. Thirteen patients, including 8 patients with FLT3/ITD mutation, achieved CR/CRi. The median overall, relapse-free, and event-free survival of the 17 patients were 1.6, 1.0, and 0.4 years, respectively [28].

1.1.1.1.4 Lestaurtinib

Lestaurtinib (CEP-701) is an orally bioavailable first generation FLT3 inhibitor. It is derived from the bacterial fermentation product K-252a as indolocarbazole alkaloid compound. Except inhibition of FLT3 Lestaurtinib also inhibits JAK2, tropomyosin receptor kinases and neurotrophin receptors [29–33]. In a phase 2 trial lestaurtinib was administered as monotherapy in untreated older patients with AML not considered fit for intensive chemotherapy, irrespective of FLT3 mutation status. This study involved 29 patients with median age of 73 years. Lestaurtinib was administered orally at doses of 60 mg and 80 mg twice daily for 8 weeks. Clinical activity was evident in 8 (30%) patients, including 3 (60%) of 5 FLT3 mutant patients and 5 (23%) of 22 evaluable FLT3 WT patients, the difference in response rates between mutation groups not reaching statistical significance. Lestaurtinib was generally well tolerated. Commonly observed toxicities included mild nausea (8 patients), emesis (5 patients), constipation (5 patients), diarrhea (6 patients), and elevations in alkaline phosphatase concentration (13 patients) [34]. Another phase 1/2 trial in 14 heavily pretreated AML patients treated with CEP-701 at an initial dose of 60 mg orally twice daily, showed clinical evidence of biologic

activity and measurable clinical response in 5 patients with significant reductions in bone marrow and peripheral blood blasts and minimal drug related toxicities [35]. A randomized assessment from UK AML 15 and AML 17 trials confirmed no statistically significant benefit observed in the combination of lestaurtinib with standard chemotherapy for newly diagnosed AML patients mostly younger than 60 years.

1.1.1.1.5 Tandutinib

Tandutinib (MLN518) is FLT3, KIT, PDGFR and type III receptor tyrosine kinases inhibitor. Tandutinib induces apoptosis and inhibits FLT3/ITD phosphorylation, cellular proliferation, and signaling of the MAPK and PI3K pathways [36]. In a phase 1 trial tandutinib was given orally (from 50–700 mg twice daily) in 40 patients with either AML or high-risk myelodysplastic syndrome (MDS) with only 8 patients with FLT3-ITD mutations. Even among the patients with FLT3-ITD mutations who were treated at potentially effective doses, response evaluation was often not possible because of rapid disease progression, sudden disease-related clinical deterioration, or tandutinib-related toxicity. Tandutinib treatment was associated muscular weakness, nausea, vomiting and less often diarrhea [37].

1.1.1.2 Second and next generation FLT3 inhibitors

1.1.1.2.1 Quizartinib

Quizartinib (AC220) is a selective and highly potent second-generation class III receptor TKI that selectively inhibits FLT3/STK1, CSF1R/FMS, SCFR/KIT, and PDGFRs in a dose dependent manner [38]. Quizartinib was first tested in phase I dose-escalation trial in patients with relapsed and refractory AML patients irrespective of FLT3 mutation status. Quizartinib was administered orally at escalating doses of 12 to 450 mg/day to 76 patients, with a median of three prior therapies and responses occurred in 23 (30%) of 76 patients, including 10 (13%) complete remissions (CR). The median duration of response was 13.3 weeks and the median survival was 14 weeks. The most common treatment-related adverse events were nausea, vomiting, and prolonged QT interval. The maximum tolerated dose (MTD) was 200 mg/day, and the dose-limiting toxicity was grade 3 QT prolongation [39]. In another 2-part, phase 1, multicenter, open-label, sequential group dose-escalation trial of quizartinib in combination with induction and consolidation chemotherapy in patients with newly diagnosed acute myeloid leukemia a total of 19 patients were enrolled. Sixteen patients (84%) achieved a response; 14 (74%) composite complete response; 2 (11%) morphologic leukemia-free state. Most common grade 3/4 adverse events were febrile neutropenia (47%), neutropenia (42%), thrombocytopenia (32%), and anemia (26%). There were no apparent additional toxicities with addition of quizartinib to chemotherapy although grade ≤ 1 QT prolongation was observed at MTD [40]. In a large phase 2 trial assigning 333 (157 in cohort 1 and 176 in cohort 2) r/r AML patients were enrolled. In cohort 1 56% of FLT3-ITD-positive patients and 36% of FLT3-ITD-negative patients achieved composite complete remission and in cohort 2 46% FLT3-ITD-positive patients achieved composite complete remission whereas 30% of FLT3-ITD-negative patients achieved composite complete remission. Across both cohorts the most common grade 3 or worse treatment-related adverse events were febrile neutropenia, anaemia, thrombocytopenia, QTcF prolongation, neutropenia, leucopenia thrombocytopenia and pneumonia [41]. Preliminary results of a randomized phase 3 study (QuANTUM-R) in patients with FLT3-ITD mutated r/r AML enrolled 367 patients, randomized 2:1 to receive quizartinib or 1 of 3 preselected investigator's

choice therapy. (low-dose cytarabine, mitoxantrone, etoposide, and intermediate-dose cytarabine or fludarabine, cytarabine, and granulocyte-colony stimulating factor with idarubicin). The results showed a significantly improved median OS for quizartinib (6.2 vs. 4.7 months; p = 0.017) and an improved cCR rate (48% vs. 27%, p = 0.0001). Rates of treatment-emergent adverse events were comparable between the 2 arms [42].

1.1.1.2.2 Crenolanib

Crenolanib (CP868596) is a benzamidine quinolone derivative, a second generation RTK inhibiting FLT3-ITD and -TKD mutations. It is potent, selective, and invulnerable to resistance-conferring kinase domain mutation. As a type I pan-FLT3 inhibitor crenolanib inhibits FLT3/WT, FLT3/ITD, FLT3-TKD, PDGFRα/β, KIT, and FLT3/D835 [43]. In nearly one third of AML patients treated with FLT3 inhibitors in different clinical trials a resistance point mutations like D835 and F691 are occurring during disease progression [44, 45]. As a more potent RTK inhibitor crenolanib could inhibit both FLT3/ITD and resistant FLT3/D835 mutants and less disruptive of erythroid colony growth, which may result in relatively lessmyelosuppression [46]. In a phase 1 trial of FLT3-ITD positive AML crenolanib was given to 69 patients divided in three cohorts, cohort A patients with R/R FLT3 AML who had not received prior FLT3 inhibitors, cohort B patients progressing on prior TKIs and cohort C patients who developed FLT3 + AML after prior MDS. Crenolanib therapy resulted in a 39% CRi and 11% PR (6 D835, 9 ITD, 3 ITD + D835) amongst the patients in cohort A with an ORR of 50% [47]. In a phase II trial, the tolerability and efficacy of crenolanib combined with standard 7 + 3 induction chemotherapy was examined in 29 patients with newly diagnosed FLT3 mutant AML. 21 of 29 (72%) patients achieved a CR after one cycle of induction with cytarabine/anthracycline/crenolanib. An additional 3 patients achieved a CR either after re-induction (1 patient) or after treatment with HiDAC or HSCT (1 patient each) [48]. In another study with the same inclusion criteria in which 26 patients were enrolled the most common advere events which led to crenolanib dose reductions were periorbital edema, delayed count recovery, LFT elevation, nausea and rash [49]. Also crenolanib was investigated in a patients with a first relapsed/primary refractory AML. The study enrolled 8 patients, received HAM followed by crenolanib. 6 patients were evaluable for responses with a complete remission rate of 67% (2 CR, 2 CRi), including 2 pts who were refractory to front line chemotherapy. 2 of 3 patients with FLT3 activating mutations (1 with ITD and 1 with D835) achieved complete remission with complete count recovery; the third pt (FLT3-ITD) had 10% residual blasts after 1 cycle of induction [50]. Ohanian et al. studied the effect of crenolanib combined with idarubicin and high-dose Ara-C in 13 pts (median age of 51 years) with multiply relapsed/refractory FLT3+ AML. The ORR in 11 pts evaluable for response was 36% (1 CR, 3 CRi; 2 not evaluable because of early discontinuation of therapy). The median OS for all patients was 259 days; median OS by prior therapies was 259 days for patients with ≤2 prior therapies, and 53 days for patients with ≥3 prior therapies. No dose-limiting toxicities were observed at any of the dose levels explored and there were no dose reductions required. Non-hematologic adverse events assessed as possibly or probably related to crenolanib were all grade 1 in severity, including: nausea, vomiting, diarrhea, and abdominal pain [51].

1.1.1.2.3 Gilteritinib

Gilteritinib (ASP2215) is a selective next-generation novel dual FLT3 (to a lesser extent to FLT3-TKD than -ITD)/AXL inhibitor. Gilteritinib was investigated

in 252 patients with relapsed or refractory acute myeloid leukaemia in one of seven dose-escalation (n = 23) or dose-expansion (n = 229) cohorts. At least 90% of FLT3 phosphorylation inhibition was seen by day 8 in most patients receiving a daily dose of 80 mg or higher. In the full analysis 249 patients were included, 8% achieved complete remission, 4% complete remission with incomplete platelet recovery, 18% complete remission with incomplete haematological recovery, and 10% partial remission. The most common grade 3–4 adverse events irrespective of relation to treatment were febrile neutropenia (39%), anaemia (24%), thrombocytopenia (13%), sepsis (11%), and pneumonia (11%) [52]. In another open-label phase 1 study in 24 Japanese patients with relapsed/refractory acute myeloid leukemia the ORR among patients with mutated FLT3 was 80% and among FLT wild-type was 36.4%. The MTD was 200 mg/d, dose-limiting toxicities were grade 3 tumor lysis syndrome and grade 3 elevated blood lactate dehydrogenase, amylase, blood creatine phosphokinase levels, and syncope [53]. The phase 3 ADMIRAL trial assessing oral gilteritinib 120 mg per day versus salvage chemotherapy in adult r/r FLT3 mutated AML patients led to an FDA approval for gilteritinib. 369 adults with FLT3 mutated AML in first relapse or refractory to front-line therapy were enrolled. The 21% of patients who achieved had a median time to response of 3.6 months [54].

1.1.1.2.4 Cabozantinib

Cabozantinib is an oral tyrosine kinase inhibitor of multiple receptor tyrosine kinases and exhibits anti-tumor activity in several cancers [55, 56]. It inhibits FLT3, MET, AXL, vascular endothelial growth factor receptor, and KIT. In a study among 18 patients with relapsed/refractory AML, 5 harboring FLT3/ITD mutations no patients had a marrow response according to formal criteria. 4 patients had peripheral blast reductions, 2 of these 4 patients transiently cleared circulating blasts, 1 patient experienced a reduction in marrow blasts, and 1 had stable disease [57] (**Table 1**).

1.2 Mutations in epigenetic modifiers: regulators of DNA methylation and chromatin modification drugs

1.2.1 IDH inhibitors

For the first time IDH1 mutations in AML were identified in 2009 by sequencing an acute myeloid leukemia genome. IDH is an enzyme that catalyzes the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (α-KG). 5-methylcytosine (5mC) coverts to 5-hydroxymethylcytosine (5hmc) as a result of interaction between α-KG and TET2 which promotes DNA and histone demethylation [58]. Approximately 8–19% of AML cases carry IDH2 mutations, with another 7–14% carrying IDH1 mutations [59]. IDH1/2 are found with higher frequency in older patients and patients with a normal karyotype [60, 61]. IDH1 mutations almost exclusively occur at R132 while IDH2 involve substitutions at R140 or R172 [62]. While IDH2-R172 may represent a distinct genomic subgroup, which mutual exclusivity with NPM1 and with a distinct DNA methylation profile [63]. Some studies showed that IDH1 and IDH2-R172 mutation may predict a worse clinical outcome especially in CN-AML, while the IDH2-R140 concomitant NPM1 mutation may be associated with better prognosis in AML [63–65]. More further studies are needed due to the conflicting data about the prognostic impact of IDH1/2 mutations in AML.

Drug Class	Mechanism of action	Agent
Signaling and kinase pathway mutations inhibitors (FLT3 tyrosine kinase inhibitors)		
First-generation FLT3 inhibitors	Inhibition of FLT3	
		Midostaurin (PKC412)
		Sorafenib
		Sunitinib
		Lestaurtinib
		Tandutinib
Second and next generation FLT3 inhibitors		
		Quizartinib
		Crenolanib
		Gilteritinib
		Cabozantinib
Mutations in epigenetic modifiers: Regulators of DNA methylation and chromatin modification drugs (IDH inhibitors)	Inhibition of IDH2	Enasidenib
	Inhibition of IDH1	Ivosidenib
Pro-apoptotic agents (Bcl-2 inhibitors)	Inhibition of BCL2	Venetoclax
Hedgehog Inhibition (Smoothened inhibitors)	inhibition of of HH/GLI signalling	Glasdegib
		Sonidegib
		Vismodegib
Polo-like kinase inhibitors	Inhibition of Plk1	Volasertib
E-selectin inhibitors	Inhibition of E-selectin	Uproleselan
Checkpoint inhibitors	Inhibition of PD-1	Nivolumab Pembrolizumab
	Inhibition of CTLA-4	Ipilimumab
CD33-targeted therapy	Inhibition of CD33	Gemtuzumab Ozogamicin
		Vadastuximab talirine

Table 1.
Selected AML targeted agents.

1.2.1.1 Enasidenib

Enasidenib (AG-221) is the first oral IDH mutation–specific inhibitor. It is a bivalent inhibitor of R140Q and R172K mutated IDH2 and induces terminal differentiation of leukemic blasts into neutrophils in vivo [66]. IDH2 inhibitor enasidenib (AG-221/CC-90007) showed promising activity as a single agent in patients with mutated IDH2 in first-in-human phase 1/2 study with 345 patients enrolled. Median age was 68 years. 214 of 345 patients (62%) with relapsed or refractory (R/R) AML received enasidenib, 100 mg/d. 19.6% attained complete remission, 10.3% proceeded to an allogeneic bone marrow transplant, and the overall response rate was 38.8%. 43.1% red blood cell transfusion–dependent and 40.2% platelet transfusion–dependent patients achieved transfusion independence. Response and survival were comparable among patients with IDH2-R140 or IDH2-R172 mutations. Among all 345 patients, the most common grade 3 or 4 treatment-related adverse events were hyperbilirubinemia (10%), thrombocytopenia (7%), and IDH differentiation

syndrome (6%) [67]. These results led to the FDA approval of enasidenib in r/r IDH2 mutated AML patients on 1 August 2017. With regard to predictors of response, the IDH2 mutation allele burden at study entry had no effect on response rate [66]. In a open-label, multicenter, phase 1 study patients 134 newlydiagnosed mIDH1 or mIDH2 AML were treated with induction therapy in combination with either ivosidenib 500 mg once daily (for mIDH1) or enasidenib (mIDH2) 100 mg daily. Among the 77 enasidenib-treated patients evaluable for efficacy, a response of CR, CRi, or CRp was achieved in 73% patients with de novo AML and in 63% patients with sAML. The most frequent co-occurring baseline mutations for patients with IDH2 mutations were DNMT3A, SRSF2 and ASXL1 [68].

1.2.1.2 Ivosidenib

Ivosidenib (AG-120) is a potent and selective IDH1 mutation small-molecule inhibitor. In phase 1, multicenter, open-label, dose-escalation and dose-expansion study 258 patients received ivosidenib orally, daily, in 28-day cycles. In the primary efficacy population (125 patients), the rate of complete remission or complete remission with partial hematologic recovery was 30.4%, the rate of complete remission was 21.6% and the overall response rate was 41.6%. The median durations of these responses were 8.2 months, 9.3 months, and 6.5 months, respectively. No residual detectable IDH1 mutations on digital polymerase-chain-reaction assay were detected in 21% patients who had a complete remission or complete remission with partial hematologic recovery. The most common adverse events (in ≥20% of the patients), irrespective of a relationship to ivosidenib, were diarrhea, leukocytosis, febrile neutropenia, nausea, fatigue, dyspnea, prolongation of the QT interval. Peripheral edema, anemia, pyrexia, cough and and differentiation syndrome [69]. The results of this study led to the FDA approval of ivosidenib in r/r IDH1 mutated AML patients on 2 may 2019. Prescribing information contains a boxed warning about the risk of differentiation syndrome which may be life-threatening or fatal. In a open-label, multicenter, phase 1 study patients 134 newlydiagnosed mIDH1 or mIDH2 AML were treated with induction therapy in combination with either ivosidenib 500 mg once daily (for mIDH1) or enasidenib (mIDH2) 100 mg daily. Among the 41 ivosidenib-treated patients evaluable for efficacy, a response of CR, CRi or CRp was achieved in 93% patients with de novo AML and 46% patients with sAML. Twenty-one patients received ≥1 cycle of consolidation therapy and 11 patients received maintenance after consolidation. Seventeen patients proceeded to HSCT. For patients with IDH1 mutations the most frequent co-occurring baseline mutations were DNMT3A, NPM1 and NRAS. MRD-negative CRs using flow cytometry were observed in 89% of patients with IDH1 positive mutational status [68].

1.3 Pro-apoptotic agents

1.3.1 Bcl-2 inhibitors

Bcl-2 gene is located on chromosome 18q21.33 and it was discovered in 1985 through cloning the breakpoint of a translocation of t(14;18) found in follicular B lymphomas [70]. Bcl-2 is an integral protein of the mitochondrial membrane but has also been identified on endoplasmic reticulum and the nuclear envelope [71]. BCL2 family members are classified into pro and anti-apoptotic proteins. The anti-apoptotic BCL2 family contains 4 proteins: BCL2, BCLXL, BCL-w, and MCL-1. Through direct activation of the effector proteins or antagonizing the effect of antiapoptotic proteins the pro-apoptotic proteins lead to an activation of caspase proteases [72]. Bcl 2 has proven to be major negative regulator in apoptosis, playing

key roles in neoplastic transformation and leukemogenesis [73]. Overexpression of anti-apoptotic BCL2 proteins such as BCL2, BCL2L1 and MCL1 is widely associated with tumour initiation, progression and chemo resistance in AML [74].

1.3.1.1 Venetoclax

Venetoclax (ABT-199/GDC- 0199) is a highly selective oral BCL2 inhibitor and does not show significant BCL-XL antagonism [75]. Venetoclax induces apoptosis in AML cell lines, in-vitro patient samples and in mouse xenograft models [75, 76]. In a phase II, single-arm study in 32 patients with high-risk relapsed/refractory AML or unfit for intensive chemotherapy venetoclax was given at a dose of 800 mg daily. The overall response rate was 19%, an additional 19% of patients demonstrated antileukemic activity not meeting IWG criteria (partial bone marrow response and incomplete hematologic recovery). Twelve (38%) patients had IDH 1/2 mutations, of whom 4 (33%) achieved complete response or complete response with incomplete blood count recovery. The responses median progression free interval was 2.5 months. Common adverse events included nausea, diarrhea, febrile neutropenia and hypokalemia. Due to potential tumour lysis syndrome as seen in chronic lymphocytic leukemia, a daily dosing ramp up of venetoclax was executed until 800 mg per day [77]. More effective results were achieved in studies where venetoclax was combined with either low-dose cytarabine (LDAC) or hypomethylating agents (HMAs). In a phase Ib/II study in previously untreated patients with AML venetoclax was combined with low-dose cytarabine in 82 adults 60 years or older. The median age was 74 years, 49% had secondary AML, 29% had prior HMA treatment, and 32% had poor-risk cytogenetic features. 54% achieved complete remission (CR)/CR with incomplete blood count recovery. The median OS was 10.1 months (95% CI, 5.7 to 14.2), and median duration of response (DOR) was 8.1 months (95% CI, 5.3 to 14.9 months). Early (30-day) mortality was 6% [78]. In another phase 1b study of venetoclax plus decitabine or azacitidine in untreated AML patients ≥65 years ineligible for standard induction therapy 145 patients were enrolled. Median age was 74 years, with poor-risk cytogenetics in 49% of patients. With a median time on study of 8.9 months, 67% of patients (all doses) achieved complete remission (CR) + CR with incomplete count recovery (CRi), with a CR + CRi rate of 73% in the venetoclax 400 mg + HMA cohort. No tumor lysis syndrome was observed. Common adverse events (>30%) included nausea, diarrhea, constipation, febrile neutropenia, fatigue, hypokalemia, decreased appetite, and decreased white blood cell count [79]. Due to these marked results venetoclax received FDA approval for combination with low dose cytarabine and HMAs. DiNardo et al. assessed the safety and efficacy of venetoclax in combination with FLAG-IDA in a heavily pre-treated r/r AML patients. Study included 12 patients, of 11 patients, 8 patients (73%) achieved a best response of CR/CRi (7 CR, 1 CRi) with a 6-month survival rate of 67%. Of the 8 responding patients, three patients proceeded to allogeneic SCT [80].

1.4 Hedgehog inhibition

1.4.1 Smoothened inhibitors

The Hedgehog (Hh) family of proteins control cell growth and survival. The Hedgehog signalling pathway (HhP) is essential for embryonic development and usually silenced in adult tissues. Germline mutations that subtly affect Hh pathway activity are associated with developmental disorders, whereas somatic mutations activating the pathway have been linked to multiple forms of human

cancer [81–83]. Aberrant activation of the HhP has been implicated in the maintenance of leukaemia stem cells in several model systems. Overexpression of various HH/GLI components have been found in chemotherapy resistant myeloid blasts and subsequent inhibition of the HH/GLI pathway revised the sensitivity to chemotherapy [84, 85].

1.4.1.1 Glasdegib

Glasdegib (PF-913) is an oral, potent, selective, small molecule inhibitor of HH/GLI signalling, which binds to the smoothened (SMO) receptor [86]. In vitro treatment with Glasdegib induced a decrease in the quiescent cell population and in vivo treatment attenuated the leukemia-initiation potential of AML cells in a serial transplantation mouse model [87]. An open-label, dose-finding, phase 1 study of glasdegib in 47 adult patients with myeloid malignancies (AML, n = 28) found 400 mg once daily as the MTD and a minor response was achieved (over 25% decrease from baseline in BM blasts) or better in more than 30% of AML patients. The most common treatment-related adverse events included dysgeusia, decreased appetite, and alopecia [88]. Based on this study a phase II, randomized, open-label, multicenter study evaluated the efficacy of glasdegib plus low-dose cytarabine (LDAC) in patients with AML or high-risk myelodysplastic syndrome unsuitable for intensive chemotherapy. Glasdegib 100 mg was administered orally in 28-day cycles. Eighty-eight and 44 patients were randomized to glasdegib/LDAC and LDAC, respectively. Median overall survival was 8.8 (6.9–9.9) months with glasdegib/LDAC and 4.9 (3.5–6.0) months with LDAC. Fifteen (17.0%) and 1 (2.3%) patients in the glasdegib/LDAC and LDAC arms, respectively, achieved complete remission ($P < 0.05$) [89]. Based on this study the FDA approved glasdegib in combination with low dose cytarabine for AML patients unfit for IC. Another phase-II study to evaluate glasdegib from day 3 plus standard-induction chemotherapy for untreated and fit AML or high-risk MDS patients revealed that 46.4% of patients achieved CR. Among all 69 patients, median OS was 14.9 (80% CI 13.4–19.3) months, with 12-month survival probability 66.6%. The most common treatment-related adverse events (≥50% patients) were diarrhea and nausea [90].

1.4.1.2 Sonidegib

Sonidegib (LDE225) is a specific SMO inhibitor and in refractory AML cells increased cell apoptosis and the efficacy of Adriamycin against tumor cells and lowered the expression of the targeted protein [91]. In a phase I/Ib study of azacitidine and sonidegib in myeloid malignancies the best response outcome for untreated AML/MDS patients was 23.1% and for rel/ref 7.1%. However, the rate of SD was remarkably high particularly in the rel/ref AML population at 76%. The most common Gr 3/4 AEs were: thrombocytopenia, neutropenia, anemia and leukopenia [92].

1.4.1.3 Vismodegib

Vismodegib (GDC-0449) safety and efficacy were evaluated in a phase Ib trial in patients with relapsed/refractory acute myeloid leukaemia. All enrolled patients had received prior cancer therapy; most had received more than one therapy, including hypomethylating agents, immunomodulators and targeted signalling pathway inhibitors. 38 received at least one dose of vismodegib but the study was terminated by the sponsor because of lack of efficacy [93].

1.5 Polo-like kinase inhibitors

Polo-like kinases (Plks) are a family of 5 highly conserved serine/threonine protein kinases. They play a key role in mitotic checkpoint regulation and cell division. Plk1 has been shown to be overexpressed in a range of human cancers, including non-small cell lung cancer, prostate, ovarian, breast, and colorectal cancer as well as AML [94–96].

1.5.1 Volasertib

Volasertib (BI 6727) is a low-molecular-weight, adenosine triphosphate–competitive kinase inhibitor that potently inhibits Plk1 [97]. In a randomized, phase 1/2, open-label, multicenter trial of low-dose cytarabine with or without volasertib in patients with AML ineligible for intensive induction therapy eighty-seven patients (median age 75 years) received LDAC or LDAC + volasertib. The result confirmed greater clinical efficacy in the combination arm, statistically significant in CR (30 vs. 13.3%, P = 0.052). Median overall survival was 8.0 vs. 5.2 months, respectively. LDAC + volasertib led to an increased frequency of adverse events that was most pronounced for neutropenic fever/infections and gastrointestinal events [98].

1.6 E-selectin inhibitors

The endothelial cell adhesion molecule E-selectin is a key component of the bone marrow hematopoietic stem cell (HSC) vascular niche regulating balance between HSC self-renewal and commitment. E-selectin is expressed transiently in the normal vasculature during an inflammatory response and constitutively in the bone marrow. E-selectin directly triggers signaling pathways that promote malignant cell survival and regeneration. In vivo AML blasts with highest E-selectin binding potential are 12-fold more likely to survive chemotherapy and main contributors to disease relapse [99, 100].

1.6.1 Uproleselan

Uproleselan (GMI-1271) is a novel antagonist of E-selectin that down-regulates cell survival pathways and enhances chemotherapy response. In a single arm phase I/II trial of 47 adults with relapsed/refractory AML were treated with GMI-1271 in combination with MEC chemotherapy. GMI-1271 was given 24 hrs prior, then every 12 hrs during and for 48 hrs post induction/consolidation. With a median follow-up of 11 months, the ph 1 median leukemia free survival was not reached and overall survival was 7.6 months. ORR (CR/CRi/MLFS/PR) was evaluable in 21 patients was 50%. Remission rate (CR/CRi) was 45%.Common Gr 3/4 AEs were febrile neutropenia, sepsis, bacteremia, hypoxia. 30 and 60 day mortality were 0 and 7%, respectively [101]. A pivotal phase 2/3 study (NCT03616470) is underway to assess the efficacy and safety of uproleselan with standard salvage chemotherapy in R/R AML. The study is a global, randomized, double-blind, phase 3 trial in adults aged 18–75 years with R/R AML and fit for chemotherapy [102].

1.7 Checkpoint inhibitors

The development of immune checkpoint inhibitors (ICIs) is a revolutionary milestone in the field of immuno-oncology. Immune checkpoint blockade removes inhibitory signals of T-cell activation, which enables tumor-reactive T cells to overcome regulatory mechanisms and mount an effective antitumor response [103–105].

Recent studies suggest a novel mechanism that tumor cells might evade host immune attack through increased expression of PD-L1 [106]. Immune checkpoint inhibitor treatment involves programmed cell death protein 1 (PD1) and cytotoxic Tlymphocyte- associated antigen 4 (CTLA4), both of which have been used in preclinical AML models [107].

1.7.1 Nivolumab

Nivolumab (BMS-936558, ONO-4538, or MDX1106) is the first-in-human immunoglobulin G4 (IgG4) PD-1 immune checkpoint inhibitor antibody that disrupts the interaction of the PD-1 receptor with its ligands PD-L1 and PD-L2, thereby inhibiting the cellular immune response [108, 109]. In a phase IB/II study of nivolumab in combination with azacytidine in patients (pts) with relapsed AML 51 patients with a median age of 69 years (range 45–90) were included. From 35 patients evaluable for response, 6 (18%) achieved complete remission (CR)/(CRi) (3 CR, 3 CRi), 5 (15%) had hematologic improvement (HI), 9 (26%) had 50% BM blast reduction,3 pts. (9%) had stable disease >6 months, and 12 (34%) had progression. In the subgroup of patients who did not receive HMA prior treatment, the superiority of new regimen was even more evident with ORR at 52–22%. The median overall survival for the 35 evaluable pts was 9.3 months (range, 1.8–14.3). Grade 3/4 and Grade 2 immune mediated toxicities were observed in 7 (14%) and 6 (12%) patients, which included pneumonitis, nephritis, transaminitis, and skin rash. Steroids took effect on 88% of the patients who suffered from drug-related toxicities [110]. A single-arm, phase 2 part of the phase 1–2 study of nivolumab in combination with idarubicin and cytarabine was conducted in 44 patients with newly diagnosed acute myeloid leukaemia or high-risk myelodysplastic syndrome. The median overall survival was 18.54 months and median event-free survival was not reached. Among the 44 evaluable patients, the ORR was 77% including 63% CR and 14% CRi. Concerning drug toxicities, the grade 3–4 adverse events were observed in six patients, including rash, colitis, pancreatitis and transaminitis [111]. Using nivolumab in post-transplantation setting showed limited efficacy. Among three relapsed AML patients after allo-HSCT treated with nivolumab, one achieved CR, one experienced stabilization, and the third failed to respond [112].

1.7.2 Pembrolizumab

Pembrolizumab (MK-3475) is another drug that blocks PD-1. In a multicenter phase II study pembrolizumab was administered after high-dose cytarabine salvage chemotherapy in 26 R/R AML patients with median age of 54. The overall response rate was 42% with 9 CR/CRi, one PR, and one patient with morphologic leukemia free state. The median OS was 10.5 months. Most frequently observed grade 3 AEs included hepatitis, rash, and epigastric pain [113]. In another single center, single arm trial of pembrolizumab followed by decitabine in 10 patients R/R AML patients with median age of 62, the ORR was 20% with one patient achieving MRD-negative CR. With a median follow-up of 13 months, the mOS was 7 months [114].

1.7.3 Ipilimumab

Ipilimumab is a human IgG1 monoclonal antibody, CTLA-4 antagonist. In a phase I/Ib, open label, multicenter study of treating patients with relapsed hematological malignancies after allo-SCT with ipilimumab 12 AML patients were enrolled. Complete response was observed in five patients (23%) and the 1-year survival rate was 49%. Immune-related adverse events occurred in three patients. Response was

associated with in situ infiltration of CD8+ T cells as well as enrichment of effector T cell subsets [115].

1.8 CD33-targeted therapy

The CD33 antigen is expressed on the blast cells (85–90%) of most cases of acute myeloid leukemia [116–118]. CD33 seems to be much less expressed on normal hematopoietic stem cells and has decreased expression during the differentiation of the myeloid lineage. Mature granulocytes do not express a significant amount of CD33. That makes CD33 an promising target for AML targeted therapy [119, 120]. The only non-haematopoietic cells expressing CD33 are hepatocytes, which explains to some extent hepatic toxicity induced by anti CD33 antibodies [121, 122].

2. Gemtuzumab ozogamicin (GO)

Gemtuzumab ozogamicin (Mylotarg) is a humanized anti-CD33 IgG4 monoclonal antibody conjugated to a cytotoxic agent N-acetyl gamma calicheamicin via an acid-labile hydrazone linker. After GO binds to CD33, calicheamicin is being released and generates single and double strand breaks with subsequent cellular death [123]. In a phase I dose escalation study of an anti-CD33 calicheamicin immunoconjugate 40 patients with relapsed or refractory CD33(+) AML were treated. Leukemia was eliminated from the blood and marrow of 8 (20%) of the 40 patients and the MTD was determined to be 9 mg/m^2 [124]. After the encouraging results from three open label phase II studies, the FDA approved GO for the treatment of patients with CD33-positive AML in first relapse who were ≥ 60 years and not suitable for intensive chemotherapy. 142 patients with AML in first relapse were enrolled in the studies with median age of 61 years. All patients received Mylotarg as a 2-hour intravenous infusion, at a dose of 9 mg/m^2, at 2-week intervals for two doses. 30% of patients obtained complete morphological remission. High incidences of myelosuppression, grade 3 or 4 hyperbilirubinemia, and elevated hepatic transaminase levels were registered [125]. In a post-approval phase III trial gemtuzumab ozogamicin was administred during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. 637 patients were randomly assigned to receive daunorubicin, cytarabine, and GO vs. standard induction therapy with daunorubicin and cytarabine alone. The CR rate was 69% for DA + GO and 70% for DA (P = 0.59). In this study, the addition of GO to induction or postconsolidation therapy failed to show improvement in CR rate, disease-free survival, or overall survival. Also addition of GO was associated with a higher early mortality during induction (5.5% vs. 1.4%). Major causes of death were fatal hemorrhage and infection. Based on these negative results GO was withdrawn from the market in 2010 [126]. In a subsequent trials different schedules of GO were investigated. Phase 3, open-label study enrolled 280 patients aged 50–70 years with previously untreated de novo AML. Patients were randomly assigned in a 1:1 ratio to standard treatment (control group) with or without five doses of intravenous gemtuzumab during induction and day 1 of each of the two consolidation chemotherapy courses. Although the CR rates were similar between the two arms, IC plus GO provided a significantly improved median event free survival (EFS) (19.6 vs. 11.9 months, p = 0.00018) and median OS (34 vs. 19.2 months, p = 0.046). Haematological toxicity, particularly persistent thrombocytopenia, was more common in the GO group than in the control group, without an increase in the risk of death from toxicity [127]. A meta-analysis of five open label, phase 3 trials comprising 3325 AML patients found that the addition of gemtuzumab ozogamicin significantly reduced

the risk of relapse and improved overall survival at 5 years without increased toxicity for GO treatment [128]. Based on these results FDA approved GO for the treatment of adults with newly diagnosed CD33-positive AML on 1 September 2017, and also approved Mylotarg for the treatment of patients aged 2 years and older with relapsed or refractory CD33-positive AML.

3. Vadastuximab talirine

Vadastuximab talirine (SGN-CD33A) is a novel anti-CD33 mAb conjugated to 2 molecules of pyrrolobenzodiazepine (PBD). After internalization vadastuximab where transported to the lysosomes where the PBD dimer is released via proteolytic cleavage of the linker, crosslinking DNAand leading to apoptosis. Vadastuximab is highly stable in circulation with relatively less off-target toxicity compared to GO [129]. In a dose-escalation phase 1 study 27 treatment naive patients with CD33 positive AML and median age of 74 years were treated with vadastuximab talirine. Of the 26 efficacy evaluable treatment naive patients, 6 patients achieved CR, 8 patients CRi, and 5 patients achieved a morphologic leukemia-free state [130]. In another phase 1 trial of vadastuximab talirine as monotherapy in patients with CD33-positive AML a total of 131 patients, median age, 73 years were enrolled. The CR + CRi rate was 28%, 50% of patients who responded achieved minimal residual disease negativity. Most AEs were consistent with myelosuppression, nonhematological included fatigue, nausea, and diarrhea [131]. Also vadastuximab talirine was added to a 7 + 3 induction therapy in a phase 1b study in 42 patients with a median age of 45.5 years. The CR/CRi rate was 78%. Twenty-three of 31 (74%) patients attaining CR or CRi achieved MRD negative status. No 30-day mortality or significant hepatotoxicity was observed [132]. Fathi et al. combined vadastuximab talirine with hypomethylating agents in patients with CD33-positive AML. Among 53 patients treated, the median age was 75 years. The CR + CRi rate was 70% and 51% of remissions with minimal residual disease-negative status by flow cytometry. The majority of adverse events were a result of myelosuppression, with some causing therapy delays [133]. A phase III trial (CASCADE, NCT02785900) comparing HMA with or without SGN-CD33A in elderly patients with newly diagnosed AML was terminated because of due to safety reasons, specifically a higher rate of deaths, including fatal infections, in the SGN33A arm versus the control arm.

4. Future perspectives

The great advances in understanding molecular mechanisms of AML as well as their prognostic significance have changed the therapeutic armamentarium against AML. The drugs discussed in this chapter and many novel molecules being evaluated in clinical trials are on their way to change the current standard of treatment in AML. Ongoing efforts to understand the heterogeneity of AML, the constantly changing genomic landscape, the mechanisms of resistance/refractoriness will be very important in the development of new drugs. The rational use of these drugs, their potency that might be improved by combining them with other modes of therapy will hopefully increase long-term benefits for patients with AML. Furthermore, the development of novel ultrasensitive methods for minimal residual disease detection will also refine the treatment decision making process and probably improve the survival rates. However, new issues such as extrapolation of the results from the clinical trials enrolling carefully selected patients to general practice, the access and cost of these new drugs must be considered in the treatment decision

process. It is a question of time whether personally tailored therapeutic era is going to be the new standard in the treatment of AML. Till then an increased enrolment of patients with AML into clinical trials evaluating the safety and efficacy of these new drugs and combinations is strongly encouraged and recommended.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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